AMENDMENTS

IN THE SPECIFICATION:

Please replace paragraph [0006] on page 2, with the following rewritten paragraph:

[0006] Certain regulatory regions in duplex DNA can transition into single stranded structures, including intrastrand quadruplex structures. These regulatory regions can form different intramolecular quadruplex conformations. One is a basket conformation, where the bridging loop runs diagonal to the two parallel loops. Another, which can be kinetically facile, is a chair conformation where the bridging loops run orthogonal to the two parallel loops and is a folded-over hairpin (*see e.g.*, U.S. patent application no. 10/407,449 filed April 4, 2003). Still another is a quadruplex conformation identified herein, which is present in transcription regulatory regions comprising the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2). It has been discovered that quadruplex DNA comprising these nucleotide sequences is biologically significant in that it regulates transcription of certain oncogenes, such as *HER-2/neu* and *c-MYB*. Identifying this biologically significant quadruplex conformation paves the way for identifying molecules that specifically interact with quadruplex structures.

Please replace paragraph [0007] on page 2, with the following rewritten paragraph:

[0007] Thus, featured herein is a method for identifying a molecule that modulates the biological activity of a native quadruplex nucleic acid, which comprises contacting a test quadruplex nucleic acid comprising the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2) with a candidate molecule, and determining the presence or absence of an interaction between the candidate molecule and the test quadruplex nucleic acid. One embodiment is a method for identifying a molecule that binds to quadruplex nucleic acid, which comprises contacting a test quadruplex nucleic acid with a candidate

molecule, where the quadruplex nucleic acid comprises the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2), and determining the presence or absence of binding between the candidate and the test quadruplex nucleic acid.

Please replace paragraph [0008] on page 2, with the following rewritten paragraph:

[0008] Also featured is a method for modulating the biological activity of a biologically significant quadruplex nucleic acid comprising the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2), which comprises contacting a system comprising quadruplex nucleic acid, such as native quadruplex nucleic acid, with a molecule which interacts with the quadruplex nucleic acid.

Please replace paragraph [0009] on page 3, with the following rewritten paragraph:

[0009] Another feature is a method for identifying a sequence capable of forming an intramolecular quadruplex monomer or intramolecular quadruplex dimer, which comprises contacting a nucleic acid having the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2) with a compound that binds to an iintramolecular quadruplex monomer and/or intramolecular quadruplex dimer, wherein the intramolecular quadruplex is a tetrad stabilized by a second planar structure in a parallel orientation to the tetrad, whereby the intramolecular quadruplex monomer or intramolecular quadruplex dimer is identified. In specific embodiments, the compound is TMPyP4 and/or telomestatin.

Please replace paragraph [0010] on page 3, with the following rewritten paragraph:

[0010] In specific embodiments of the methods described above, the nucleic acid is DNA, and includes the nucleotide sequence $(GGA)_3GGX_n(GGA)_3GG$, where n is 0, 1, 2, 3, 4 or 5-10 (SEQ ID NOS:3-13). In other embodiments, the nucleic acid consists of the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1), $(GGA)_3GG$ (SEQ ID NO:2) or $(GGA)_3GGX_n(GGA)_3GG$, where n is n is 0, 1, 2, 3, 4 or 5-10 (SEQ ID NOS:3-13). In an embodiment, the nucleic acid is capable of forming an intramolecular heptad/tetrad quadruplex monomer or an intramolecular heptad/tetrad quadruplex dimer.

Please replace paragraph [0011] on page 3, with the following rewritten paragraph:

[0011] Also featured herein is a method for identifying native nucleotide sequences capable of forming a quadruplex structure that modulates a biological activity, which comprises searching a database comprising nucleotide sequence information for those that include the subsequence $(GGA)_4$ (SEQ ID NO:1), the subsequence $(GGA)_3GG$ (SEQ ID NO:2), or the subsequence $(GGA)_3GGX_n(GGA)_3GG$, where n is an integer between 1 and 3 (SEQ ID NOS:4-6), and identifying a subset of the nucleotide sequences in the database comprising one of these subsequences located adjacent to an end of an open reading frame of a gene.

Please replace paragraph [0012] on page 3, with the following rewritten paragraph:

[0012] Figure 1 depicts tetrad/heptad quadruplex conformations formed by nucleotide sequences comprising the nucleotide sequences $(GGA)_4$ (SEQ ID NO:1), $(GGA)_3GG$ (SEQ ID NO:2) or $(GGA)_3GGX_n(GGA)_3GG$, where n is n is 0, 1, 2, 3, 4 or 5-10 (SEQ ID NOS:3-13). The figure also shows structures of compounds that bind to such quadruplexes.

Please replace paragraph [0013] on page 3, with the following rewritten paragraph:

[0013] Figure 2 (SEQ ID NO:14) shows nucleotide moieties in a *c-MYB* nucleotide sequence that have been mutated.

Please replace paragraph [0014] on page 4, with the following rewritten paragraph:

[0014] It has been discovered that nucleic acids comprising the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2) can form biologically relevant quadruplex structures. One example of the biological relevance of such quadruplexes is the regulation of oncogene transcription. These findings lead the way to assays useful for identifying molecules that interact with such biologically relevant quadruplex structures, as well as methods for identifying and/or distinguishing related quadruplex structures formed from those sequences. These findings also are useful for methods of identifying sequences in a database that form such structures.

Please replace paragraph [0017] beginning on page 4, with the following rewritten paragraph:

[0017] It has been discovered that nucleic acids comprising the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2) can form biologically relevant quadruplex structures. These nucleic acids adopt a quadruplex structure that differs from those reported for the *c-MYC* sequence, which adopts a biologically relevant chair conformation. The quadruplex structure formed by these sequences comprises a tetrad stabilized by second planar structure that is in a parallel orientation to the tetrad. The second planar structure includes five or more nucleotides in the nucleic acid and thereby forms a structure that is larger than a tetrad. For example, the second planar structure can contain five, six, seven, eight, nine, or ten nucleotides to form a pentad, hexad, heptad, octad, nonad, or dectad, respectively. Examples of heptad/tetrad quadruplex structures formed by nucleic acids having GGA repeats are shown in Figure 1. Determining whether a nucleic acid having the nucleotide sequence $(GGA)_4$ (SEQ ID NO:1) or the nucleotide sequence $(GGA)_3GG$ (SEQ ID NO:2) forms a quadruplex structure can be accomplished using methods described herein, such as by chemical footprinting methods, polymerase arrest analysis, structural spectrometric techniques and chemical binding techniques.

Please replace paragraph [0022] beginning on page 6, with the following rewritten paragraph:

[0022] In certain embodiments, nucleic acids utilized in the assays for identifying quadruplex-interacting molecules comprise or consist of a native nucleotide sequence. Native quadruplex DNA is a subsequence of cellular genomic DNA. The quadruplex DNA may be derived from genomic DNA from a cell of an organism, and often it is derived from genomic DNA of a human cell. Quadruplex DNA has been located, for example, in telomeres and in duplex DNAs that regulate gene transcription. Thus, the biological activity of quadruplexes includes regulation of gene transcription. Provided herein are quadruplex structures that are biologically relevant as they regulate gene transcription, particularly regulation of oncogenes. For example, quadruplexes are located in duplex DNA regions that regulate transcription of the genes *c-MYB, HER-2/neu, EGFR, c-PIM, VAV, c-SRC* and *HMGA2*, for example. Native quadruplex DNA may comprise or consist of the following nucleotide sequences having GGA

repeats: $(GGA)_4AGA(GGA)_3GGC$ (*c-MYB*) (SEQ ID NO:15); $(GGA)_4$ (*VAV*) (SEQ ID NO:1); AGAGAAGAGG$(GGA)_5$GAGG AGGAGGCGC (*HMGA2*) (SEQ ID NO:16); GGAGGGGGAGGGG (human *c-PIM*) (SEQ ID NO:17); AGGAGAA$(GGA)_2$GGT $(GGA)_3G_3$ (*HER2/neu*) (SEQ ID NO:18); $(GGA)_3$AGAATGCGA$(GGA)_2$ G_3AGGAG (*EGFR*) (SEQ ID NO:19); CCGAA$(GGA)_2A(GGA)_3G_4$ (*c-SRC*) (SEQ ID NO:20); AGCGA$(GGA)_8$GAGGAA (SEQ ID NO:21) (osteonectin/SPARC, within -92 to -57 of the open reading frame; Oncogene 26 Jun 2003, 22:4047-4061); AGAAGAG$(GGA)_3G$ (SEQ ID NO:22) (*IL-10*, within -89 to -77 of the open reading frame; J Immunology 1 July 2000, 165:286-91); GGA A $(GGA)_3$ (SEQ ID NO:23) (Decay Accelerating Factor (*DAF*); within -310 to - 290 of the open reading frame; complementary sequence is CTCCTCCTC CTTCCCCTCCCC (SEQ ID NO:24); Proc Natl Acad Sci 1991, 88:4675); and $(GGA)_2CCGA(GGA)_2$ (SEQ ID NO:25) (*WT1*; within -86 to -51 of the open reading frame; complementary sequence is $(CCT)_2GGCT(CCT)_2$ (SEQ ID NO:26); J Biol Chem 31 Jan 1997, 72:2901-2913). While quadruplex forming sequences typically are identified in regulatory regions upstream of a gene (*e.g.*, a promoter or a 5′ untranslated region (UTR)), quadruplex forming sequences also may be identified within a 3' UTR or within an intron or exon of a gene.

Please replace paragraph [0023] beginning on page 7, with the following rewritten paragraph:

[0023] In some embodiments, test quadruplex DNA sometimes has a substantially similar nucleotide sequence to a native quadruplex DNA sequence, and often has a nucleotide sequence identical to the native quadruplex DNA sequence. A similar nucleotide sequence allows for some modifications to the native sequence so long as the test DNA is capable of adopting a quadruplex conformation, which routinely can be determined by methods described herein. Test quadruplex DNA often includes a nucleotide sequence which conforms to the motif $(GGA)_4$ (SEQ ID NO:1) or $(GGA)_3GG$ (SEQ ID NO:2) where G is guanine and A is adenine. Test quadruplex DNA may include one or more flanking nucleotides on the 5' and/or 3' end of the quadruplex which are part of the quadruplex structure or not part of the quadruplex structure. As noted above, a given nucleotide sequence can be probed as to whether it forms a quadruplex structure by carrying out chemical footprinting and polymerase arrest analyses, for example, which are discussed herein.

Please replace paragraph [0028] on page 9, with the following rewritten paragraph:

[0028] Also, sequence motifs described herein may be used as "query sequences" to perform a search against public databases to identify nucleotide sequences capable of forming quadruplex structures. In certain embodiments, the query sequences are $(GGA)_4$ (SEQ ID NO:1), $(GGA)_3GG$ (SEQ ID NO:2) or $(GGA)_3GGX_n(GGA)_3GG$, where n is an integer between 1 and 3 (SEQ ID NOS:4-6), and nucleic acid comprising the nucleotide sequence is capable of forming a tetrad/heptad quadruplex structure. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al., J. Mol. Biol.* 215:403-410 (1990). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleotide sequences from Figure 1. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul, *et al., Nucleic Acids Res.* 25(17):3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (*see,* http address www.ncbi.nlm.nih.gov).

Please replace paragraph [0052] beginning on page 17, with the following rewritten paragraph:

[0052] A polymerase arrest assay is useful for determining whether transcription is modulated by a candidate molecule and/or a nucleic acid binding protein. Such an assay includes a template nucleic acid, which often comprises a quadruplex forming sequence, and a primer nucleic acid which hybridizes to the template nucleic acid 5' of the quadruplex-forming sequence. The primer is extended by a polymerase (*e.g.*, Taq polymerase), which advances from the primer along the template nucleic acid. In this assay, a quadruplex structure can block or arrest the advance of the enzyme, leading to shorter transcription fragments. Also, the arrest assay may be conducted at a variety of temperatures, including 45°C and 60°C, and at a variety of ion concentrations. An example of the Taq polymerase stop assay is described in Han, *et al.*, *Nucl. Acids Res.* 27:537-542 (1999), which is a modification of that used by Weitzmann, *et al.*, *J. Biol. Chem.* 271, 20958–20964 (1996). Briefly, a reaction mixture of template DNA (50 nM),

Tris•HCl (50 mM), $MgCl_2$ (10 mM), DTT (0.5 mM), EDTA (0.1 mM), BSA (60 ng), and 5′-end-labeled quadruplex nucleic acid (~18 nM) is heated to 90°C for 5 minutes and allowed to cool to ambient temperature over 30 minutes. Taq Polymerase (1 μl) is added to the reaction mixture, and the reaction is maintained at a constant temperature for 30 minutes. Following the addition of 10 μl stop buffer (formamide (20 ml), 1 M NaOH (200 μl), 0.5 M EDTA (400 μl), and 10 mg bromophenol blue), the reactions are separated on a preparative gel (12%) and visualized on a phosphorimager. Adenine sequencing (indicated by "A" at the top of the gel) is performed using double-stranded DNA Cycle Sequencing System from Life Technologies. The general sequence for the template strands is TCCAACTATGTATAC-INSERT-TTAGCGACACGCAATTGCTATAGTGAGTCGTATTA (SEQ ID NOS:27-28). Bands on the gel that exhibit slower mobility are indicative of quadruplex formation.

Please replace paragraph [0059] on page 21, with the following rewritten paragraph:

[0059] Thus, provided herein are methods for reducing cell proliferation or for treating or alleviating cell proliferative disorders, which comprise contacting a system having a nucleic acid comprising a native quadruplex with a candidate molecule identified herein. The system sometimes is a group of cells or one or more tissues, and often is a subject in need of a treatment of a cell proliferative disorder. A subject often is a mammal such as a mouse, rat, monkey, or human. One embodiment is a method for treating a cell proliferative disorder associated with disregulation of a gene having a $(GGA)_4$ (SEQ ID NO:1) or $(GGA)_3GG$ (SEQ ID NO:2) sequence or in proximity to a $(GGA)_4$ (SEQ ID NO:1) or $(GGA)_3GG$ (SEQ ID NO:2) sequence, such as *c-MYB, HER-2/neu, EGFR, c-PIM, VAV, c-SRC, HMGA2,* osteonectin/SPARC, *IL-10, DAF* and/or *WT1*.

Please replace paragraph [0075] on page 26, with the following rewritten paragraph:

[0075] The following examples were performed in part using single stranded DNA templates representing promoter regions of the *HER-2/neu* and *c-MYB* oncogenes. The *HER-2/neu* oncogene promoter contains a 28 base pair homopurine/homopyrimidine tract characterized by multiple GGA trinucleotide repeats. Similarly, the *c-MYB* oncogene promoter contains a 54 base pair homopurine/homopyrimidine tract also characterized by multiple GGA trinucleotide repeats. The sequence of purine rich oligonucleotides representing the purine rich strands of the *HER-2/neu* and *c-MYB* promoters are illustrated in this figure. The quadruplex forming regions described herein are highlighted and share an 11 nucleotide sequence motif: GGAGGAGGAGG (SEQ ID NO:2). The *HER-2/neu* promoter contains one of these motifs, and the *c-MYB* promoter contains three of these motifs.

Please replace paragraph [0077] on page 27, with the following rewritten paragraph:

[0077] Several nucleic acids are utilized in the examples below. One nucleic acid is HNP36 (named for *HER-2/neu* promoter, 36-mer), which contains a polypurine tract from a region located at positions -218 to -245 relative to the translation start site of *HER-2/neu* and the 4 flanking bases on either side of the tract. The HNP36 nucleic acid consists of the sequence 5'-TCACAGGAGAAGGAGGAGGTGGAGGAGGAGGGCTGC-3' (SEQ ID NO:29). Another *HER-2/neu* nucleic acid utilized for polymerase arrest assays is designated HNP86 (*HER-2/neu* promoter, 86-mer), which contains the polypurine tract of *HER-2/neu* inserted into a common sequence used for such assays, and consists of the sequence 5' -
TCCAACTATGTATACTCACAGGAGAA GGAGGAG
GTGGAGGAGGAGGGCTGCTTAGCGGCACGCAATTGCTATAGT GAGTCGTATTA-3' (SEQ ID NO:30). Another nucleic acid is designated *c-MYB* 66, a 66-mer representing the polypurine tract of the *c-MYB* promoter: 5' -TTTCTCAGGAGAAAGAGGAGGAGGAGGA

GGTCACGGAGGAGGAGGAGGAGAAGGAGGAGGAGGAGGAAACAGGTT-3' (SEQ ID NO:31). A *c-MYB* sequence utilized for polymerase arrest assays is a 100-mer that includes a cassette commonly utilized for such assays and has the sequence: 5' - TCCAACTATGTATACTTTCTCAGGA GAAAGAGGAGGAGGAGGAGGTCACGGAGGAGGAGGAGGAGAAGGAGGAGGA GGAGGAAACAGGTTTTAGCGACATTGCTATAGTGAGTCGTATTA-3' (SEQ ID NO:32).

Please replace paragraph [0082] beginning on page 28, with the following rewritten paragraph:

[0082] To further identify the DNA secondary structures formed in the EMSA analysis, the individual bands from the EMSA were excised and probed with DMS. In the presence of potassium, the *HER-2/neu* promoter gave rise to a single footprint at the $(GGA)_3GG$ (SEQ ID NO:2) sequence. The *HER-2/neu* footprint also contained three hypersensitive adenines corresponding exactly to the adenine bases postulated to be involved in the G:A:G:A:G:A:G heptad of the heptad:tetrad structure. The *c-MYB* promoter produced two potassium dependent footprints corresponding to two sets of $(GGA)_3GG$ (SEQ ID NO:2) sequences in the *c-MYB* promoter sequence. Two of three identical regions having these GGA triplet repeats produced a footprint. The EMSA analysis and DMS footprinting studies of the purine rich tract of the *HER-2/neu* promoter were indicative of a potassium dependent quadruplex. This quadruplex is formed by the GGA trinucleotide repeats with the sequence of GGAGGAGGAGG (SEQ ID NO:2). Formulation of the quadruplex was abrogated by methylation of the N7 position of the guanines and was characterized by markedly hypersensitive adenines between protected guanines.

Please replace paragraph [0083] on page 29, with the following rewritten paragraph:

[0083] An NMR study of a GGA triplet repeat oligonucleotides published by Matsugami *et al., supra,* reported that oligonucleotides containing four GGA triplet repeats formed a quadruplex in the presence of potassium ions, which consisted of a guanine tetrad stacked onto a guanine-adenine heptad. The sequence of oligonucleotide having the quadruplex DNA structure was GGAGGAGGAGG (SEQ ID NO:2), which may be characterized by the motif $(GGA)_3GG$ (SEQ ID NO:2). The position of the adenine bases in the heptad exposes the N3 position and likely accounts for their hypersensitivity to DMS. The structure on the bottom left indicates that two heptad/tetrad structures can stack onto one another to form a tetrad:heptad:heptad:tetrad dimer, and this stacking interaction of two intramolecular quadruplexes likely accounted for the slower mobility of the *HER-2/neu* promoter. The data are consistent with the formation of a heptad/tetrad quadruplex structure in the *HER-2/neu* promoter, and this structure represents a novel molecular target for the sequence selective recognition of genes containing this GGA repeat motif.

Please replace paragraph [0084] on page 29, with the following rewritten paragraph:

[0084] The *c-MYB* promoter also was studied with EMSA and DMS footprinting analysis. These studies indicated that two of the three regions of the *c-MYB* promoter containing the sequence GGAGGAGGAGG (SEQ ID NO:2) were capable of undergoing quadruplex formation, which are illustrated as regions I and II (*see e.g.*, Figure 2 for these regions). In contrast to the *HER-2/neu* promoter, the EMSA analysis did not demonstrate the presence of a slower migrating species in the presence of potassium. In the EMSA analysis, the lack of a band having altered mobility can be explained by dissociation during electrophoresis. The intramolecular stacking of two adjacent regions of heptad:tetrad formation in the *c-MYB*

promoter could prevent the formation of intermolecular heptad/tetrad multimers, as seen in the *HER-2/neu* promoter EMSA.

Please replace paragraph [0086] on page 30, with the following rewritten paragraph:

[0086] In the DNA polymerase arrest assay, the purine rich strand of the *HER-2/neu* promoter was placed into an 86 nucleotide DNA template for primer extension by taq DNA polymerase. An example of the Taq polymerase stop assay used in the study is described in Han *et al., Nucl. Acids Res. 27*: 537-542 (1999), which is a modification of that used by Weitzmann *et al., J. Biol. Chem. 271*, 20958–20964 (1996). Briefly, a reaction mixture of template DNA (50 nM), Tris·HCl (50 mM), $MgCl_2$ (10 mM), DTT (0.5 mM), EDTA (0.1 mM), BSA (60 ng), and 5′-end-labeled 18-mer template (~18 nM) was heated to 90 °C for 5 min and allowed to cool to ambient temperature over 30 min. Taq Polymerase (1 µl) was added to the reaction mixture, and the reaction was maintained at a constant temperature for 30 minutes. Following the addition of 10 µl stop buffer (formamide [20 ml], 1 M NaOH [200 µl], 0.5 M EDTA [400 µl], 10 mg bromophenol blue), the reactions were separated on a preparative gel (12%) and visualized on a phosphorimager. Adenine sequencing was performed using double-stranded DNA Cycle Sequencing System from Life Technologies. The general sequence for the template strands was TCCAACTATGTATAC-***INSERT***-TTAGCGACACGCAATTGCTATAGTGAGTCGTATTA (SEQ ID NOS:27-28).

Please replace paragraph [0091] on page 31, with the following rewritten paragraph:

[0091] A series of DMS footprinting reactions of the native and mutated *c-MYB* promoters indicated that each of the GGAGGAGGAGG (SEQ ID NO:2) quadruplex forming units were capable of undergoing quadruplex formation, if the third quadruplex forming unit was mutated. The data suggested that each of the three individual units in the native *c-MYB* promoter

underwent quadruplex formation, and the region II and III units were most favored at equilibrium and formed a tetrad:heptad:heptad:tetrad stack.

Please replace paragraph [0098] on page 33, with the following rewritten paragraph:

[0098] The effects of cell treatment on *c-MYB* expression were compared to the effects of treatment on *HER-2/neu* expression, to evaluate the role of the T:H:H:T higher order structure in selective recognition by small molecules. Because adjacent (GGAGGAGGAGG) (SEQ ID NO:2) elements are capable of stacking to create a TmPyP4 binding site *in vitro*, it was expected that TmPyP4 suppress *c-MYB* but not *HER-2/neu* expression. While *c-MYB* is expressed in hematopoietic precursor cells and abnormal overexpression is generally related to hematopoietic malignancies, aberrant expression and even amplification of *c-MYB* is also seen in certain solid tumors, such as colon cancer and some breast cancers. Evolving evidence suggests a role for the expression of *c-MYB* in colon carcinogenesis. *HER-2/neu* also is expressed in some colon carcinomas and cell lines. Colo 205 is a colon cancer cell line that co-expresses *HER-2/neu* and *c-MYB*. Colo 205 cells are treated with telomestatin and TmPyP4 for analysis of changes in both *HER-2/neu* and *c-MYB* expression, using beta-actin and GAPDH as a control.